

读书报告

吉伟利

2018-04-14

Synergistic hydrolysis of xylan using novel xylanases, β -xylosidases, and an α -L-arabinofuranosidase from *Geobacillus thermodenitrificans* NG80-2

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使用来自 *Geobacillus thermodenitrificans* NG80-2 的新木聚糖酶,
 β -木糖苷酶和 α -L-阿拉伯呋喃糖苷酶 **协同水解木聚糖**

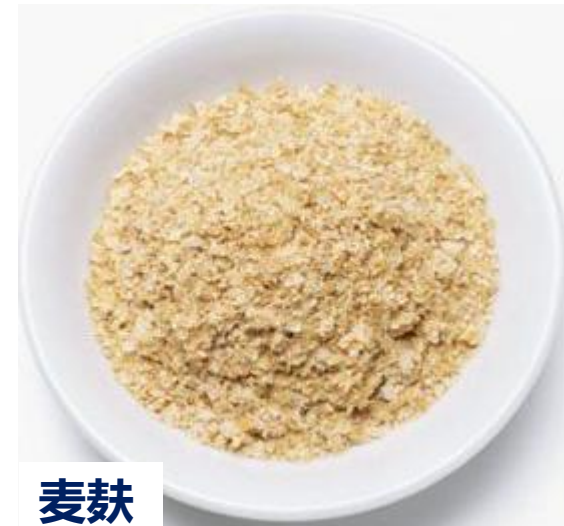
Introduction



甘蔗渣



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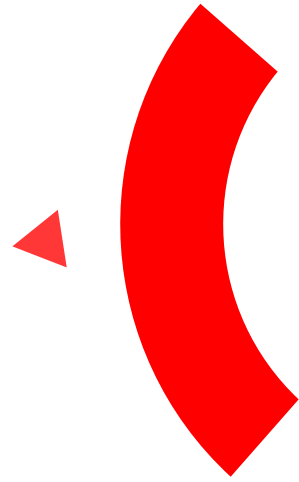
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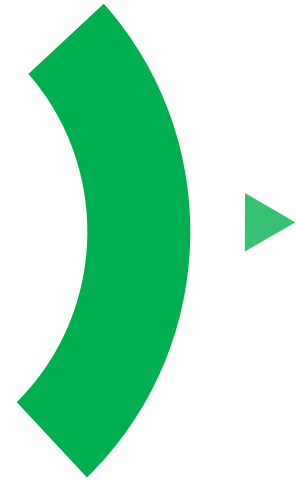
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chemical method

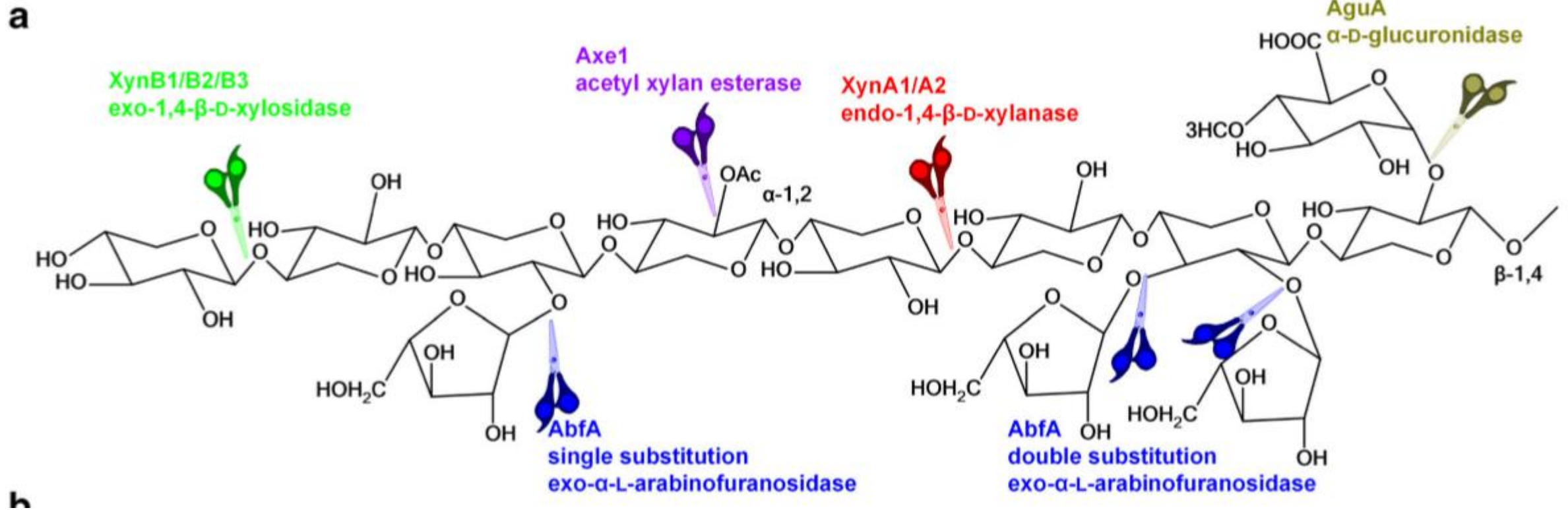


Xylan
木聚糖



enzyme hydrolysis approach

Introduction



木聚糖由被L-阿拉伯呋喃糖，甲基-D-葡萄糖醛酸和乙酰基侧链取代的β-1,4-连接的吡喃木糖组成。

Introduction

Xylanase and β -xylosidase have been reported in many microorganisms

Halorhabdus utahensis

Geobacillus stearothermophilus

Bacillus thermantarcticus

Thermomonospora fusca

Humicola insolens



mesophilic(嗜温)?



neutrophilic(中性)?

55-70°C

碱性pH



***Geobacillus thermodenitrificans* NG80-2**

来源: deep oil reservoir in Northern China

生长温度: 45~73°C之间

可以有效地将木聚糖用作其唯一的碳源和能源

two xylanases: **XynA1** (*GTNG_1761*)、**XynA2** (*GTNG_1774*)

three β -xylosidases: **XynB1** (*GTNG_1769*)、**XynB2** (*GTNG_1775*)、**XynB3** (*GTNG_1758*)

one α -L-arabinofuranosidase gene: **AbfA** (*GTNG_1791*)

Materials

primers

DNA purification kits

DNA Polymerase (聚合酶)

restriction endonucleases

T4 DNA ligase

Xylan from corn cob, oat spelt, birchwood, beechwood, and barley β -glucan, CMC-Na, xylose, pNP-Xyl, pNP-Gal, pNP-Glc, pNP-Araf, pNP, PMSF
Sugar beet arabinan, water-soluble wheat arabinoxylan, debranched arabinan, xylobiose, xylotriose, xylotetraose, and xylopentose were purchased from Megazyme

Materials and methods

Bacterial strains, plasmids, and growth conditions

Bacteria: *G. thermodenitrificans* NG80-2

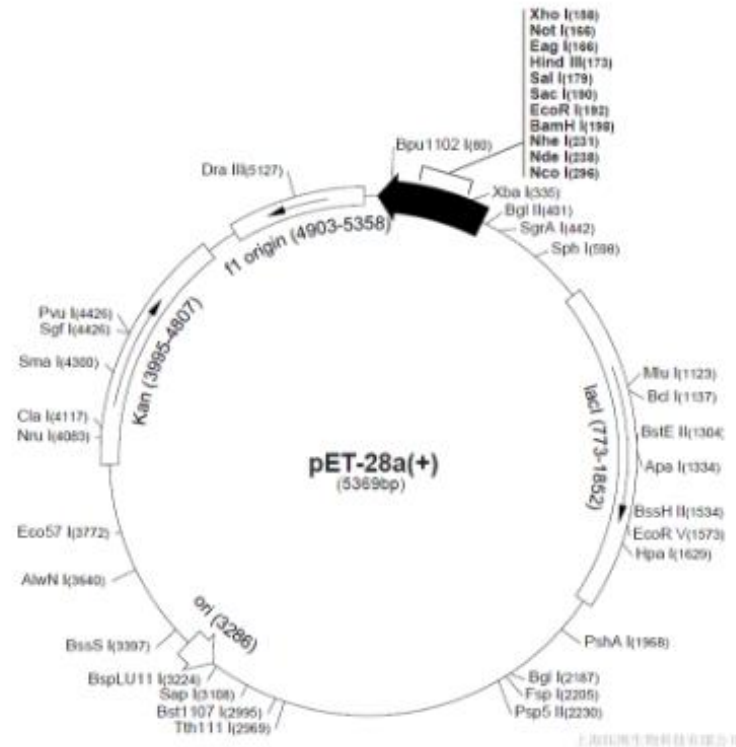
60°C

E.coli DH5α and BL21(DE3)

37°C

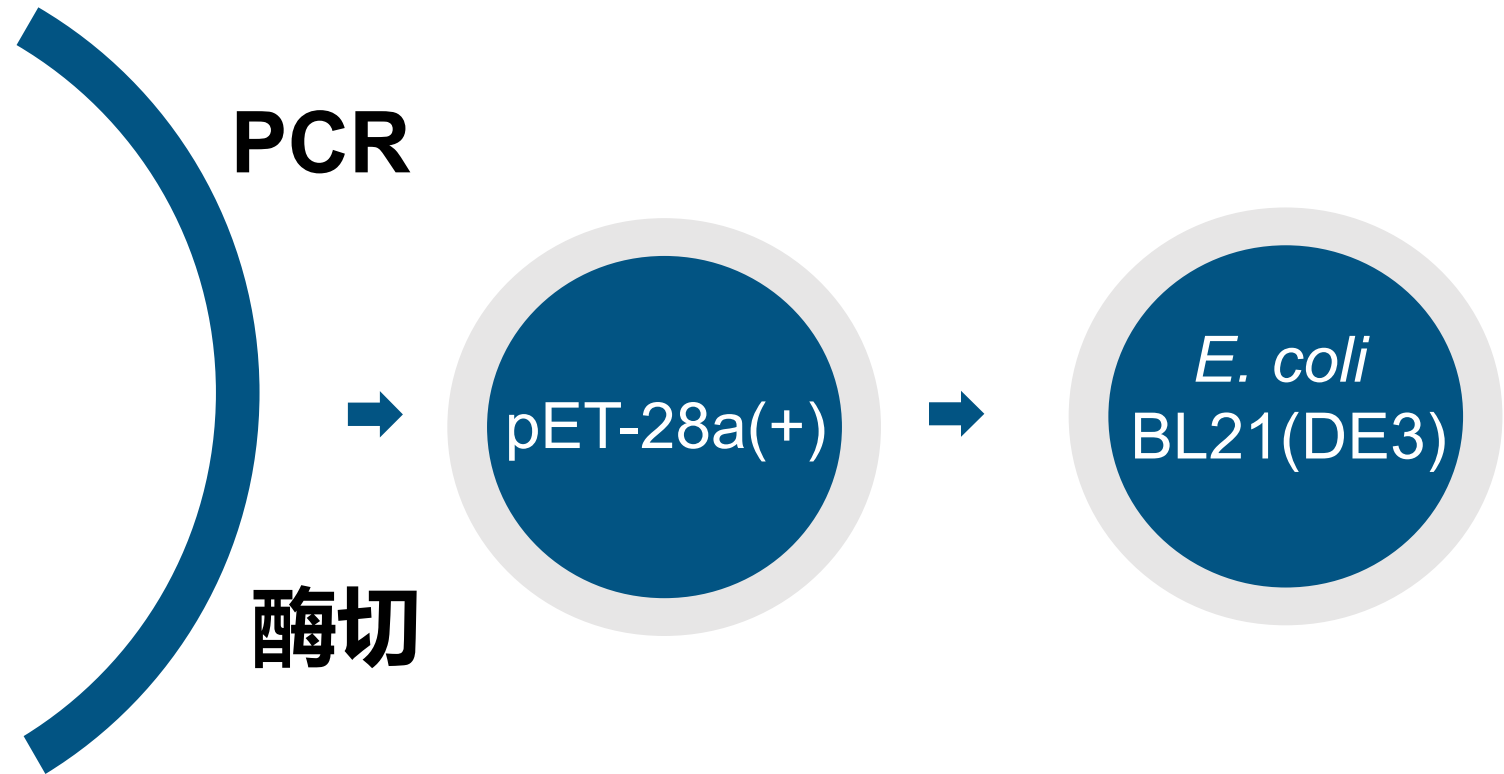
plasmids : pET-28a(+)

Kanamycin (50 mg/mL)



Construction of recombinant plasmids

- GTNG_1761 *XynA1*
- GTNG_1774 *XynA2*
- GTNG_1769 *XynB1*
- GTNG_1775 *XynB2*
- GTNG_1758 *XynB3*
- GTNG_1791 *AbfA*



Materials and methods

Protein expression and purification

1、OD₆₀₀ reached 0.6

0.1mM isopropylthio- β -D-galactopyranoside(半乳糖) for 4h at 37°C

2、10,000×g for 10 min at 4°C

washed with binding buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 10 mM imidazole)

resuspended in the binding buffer (1 mM PMSF and 1 mg/mL of lysozyme)

3、ultrasonic processor (超声破碎)

10 mg/L RNase A and 5 mg/L DNase I and centrifuged at 18,000×g for 30min

4、Fast Flow column eluted

20 mM imidazole 250 mM imidazole

5、SDS-PAGE and native-PAGE

Materials and methods

Enzyme activity assay

DNS

100 μ L total volume

for 10 min at 60 °C

150 μ L of DNS solution

to a 100°C water bath for 10 min

and then cooled to ambient temperature

UV-2550 UV-Vis spectrophotometer

540nm

performed separately in triplicate



Materials and methods

Biochemical properties of the enzymes

Optimum temperature

Temperature range 25 to 100 °C at pH 7.0 for 20 min

Optimum pH

for 20 min at pH values from 3.0 to 12.0 in universal buffer at the optimal temperature

Thermostability

50 to 85°C

various metals

(5 mM Na⁺, K⁺, Cu²⁺, Ca²⁺, Co²⁺, Ni²⁺, Mn²⁺, Mg²⁺, Zn²⁺, Fe³⁺, or Al³⁺)

inhibitors

(5mM DTT, βME, EDTA)

surfactants (0.5% SDS or Tween40) organic solvents (5% alkane or alcohol)

Tolerance to xylose by β -xylosidase (β -木糖苷酶对木糖的耐受性)

The influence of various xylose concentrations on the β -xylosidase activities of XynB1, XynB2, and XynB3 was assessed

The substratep NP-Xyl, appropriate amounts of enzymes, and various concentrations of xylose (50–1000 mM) were mixed together and incubated at 50 °C for 10 min.

Materials and methods

Activity of xylanase, β -xylosidase, and α -L-arabinofuranosidase combinations

Synergetic activity of xylanase, β -xylosidase, and α -L-arabinofuranosidase on various substrates,

including birchwood xylan, beechwood xylan, and oat spelt xylan, was tested as described previously

0.25U XynA1 or XynA2, 1.0 U XynB1 or XynB2 or XynB3, and/or 5.0 U AbfA)

and 900 μ L of 0.5%(w/v) substrate in 100mM citrate– sodium citrate buffer (pH 6.0),

were incubated **at 50 °C for 12 h**

The reactions were terminated in a 100 °C water bath for 10min and cooled to room temperature



Materials and methods

Analysis of sugar compositions by HPLC

at 10,000 rpm, 4 °C for 10 min

HPLC

Bioinformatics

利用生物信息学 对基因组进行对比分析
氨基酸分析
信号肽预测等

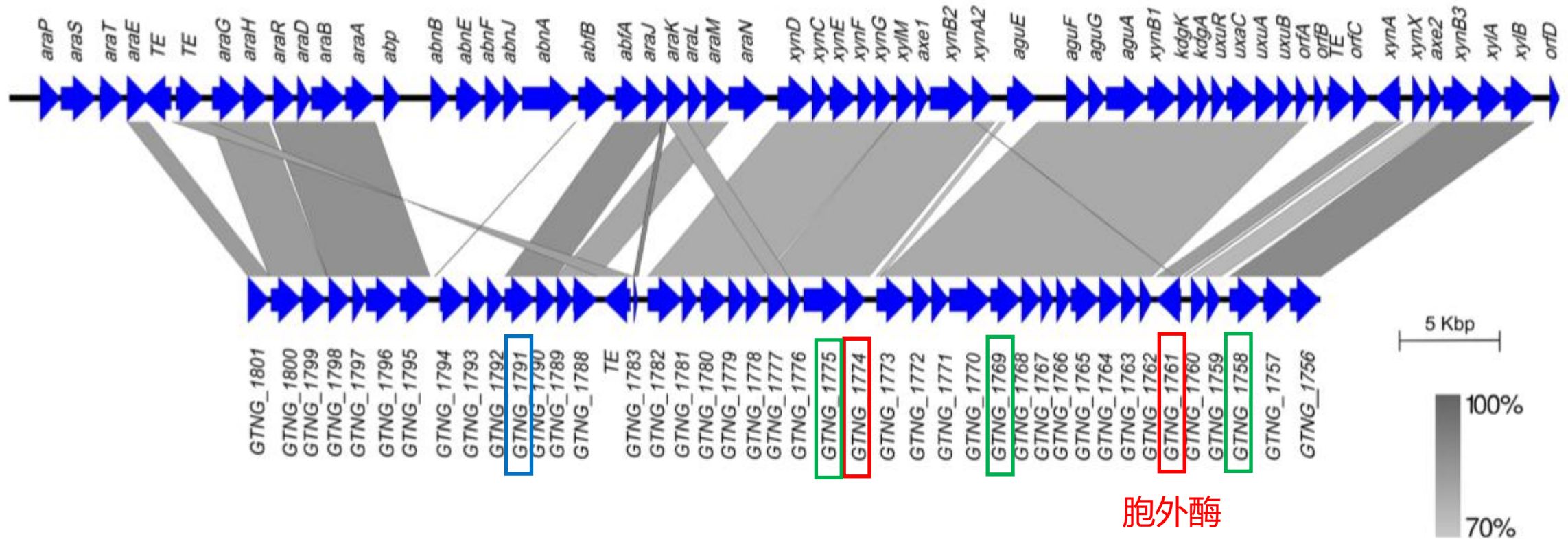
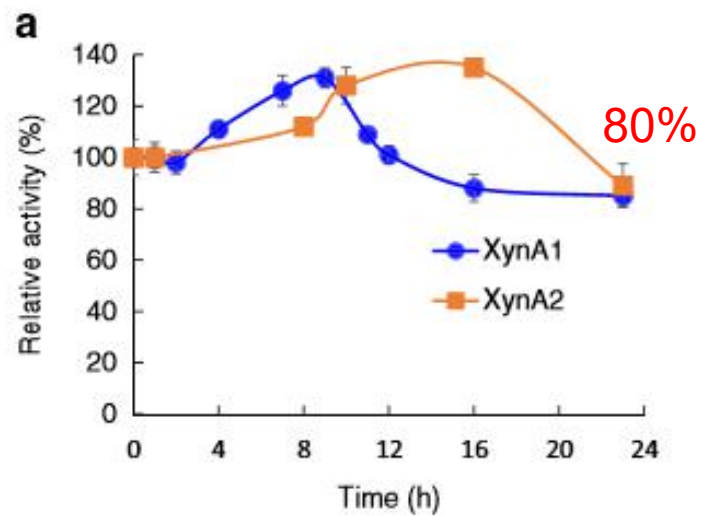
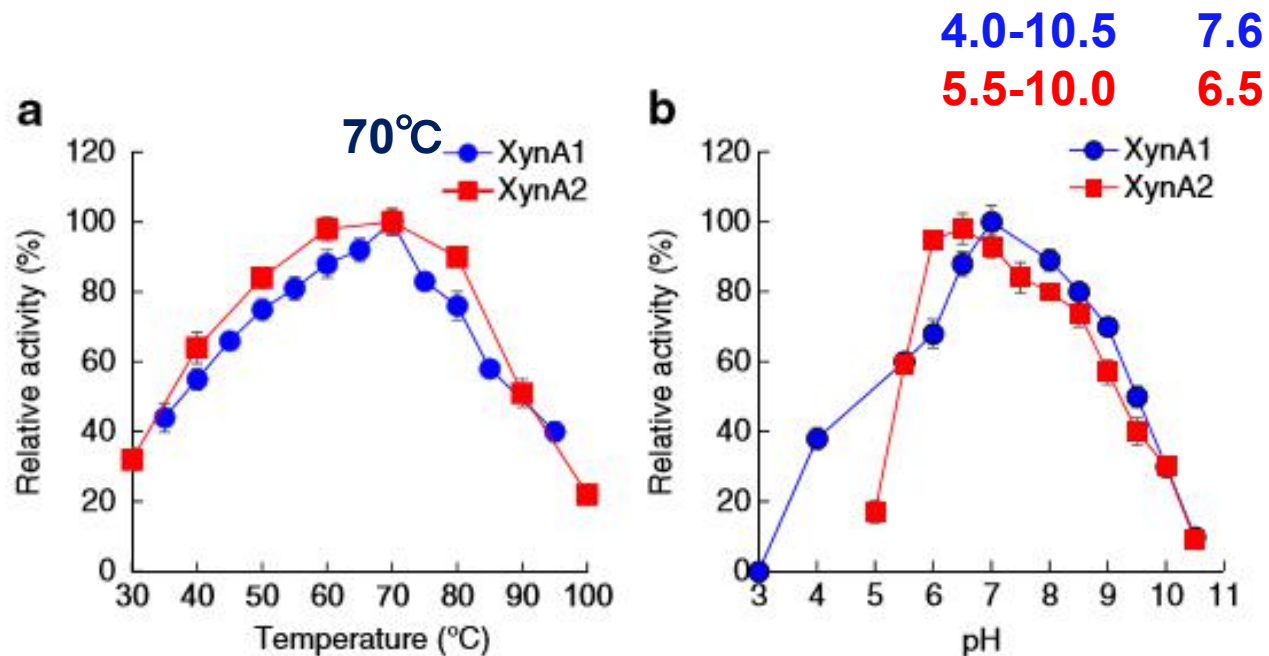


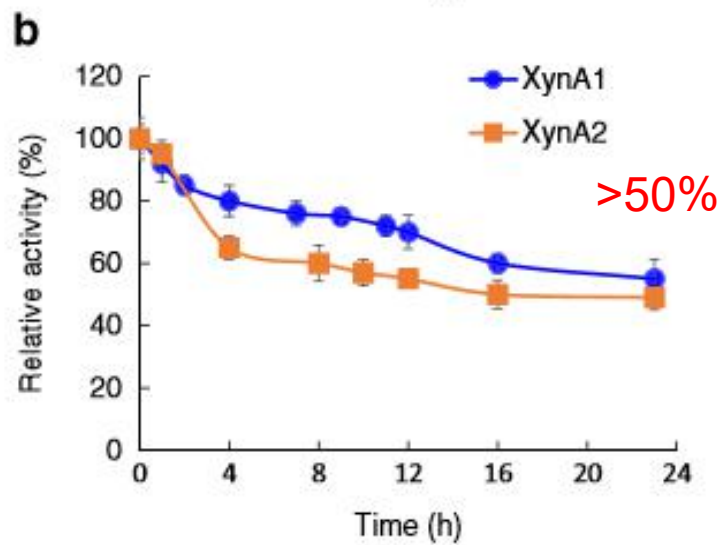
Fig. 2 Comparison of the genes involved in xylan hydrolysis in *G. thermodenitrificans* NG80-2 and *G. stearothermophilus* T-6. Comparisons were made using tBLASTx, and similarities with E values lower than 0.01 are plotted as *gray lines*, as indicated by the figure legend

XynA1	48kDa	}	GH10
XynA2	40kDa		
XynB1	62kDa		GH39
XynB2	83kDa		GH52
XynB3	64kDa		GH43
AbfA	59kDa		GH51

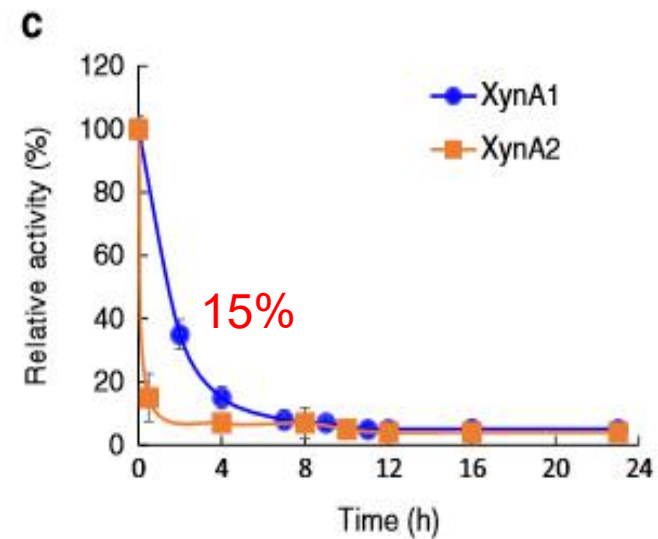
Results



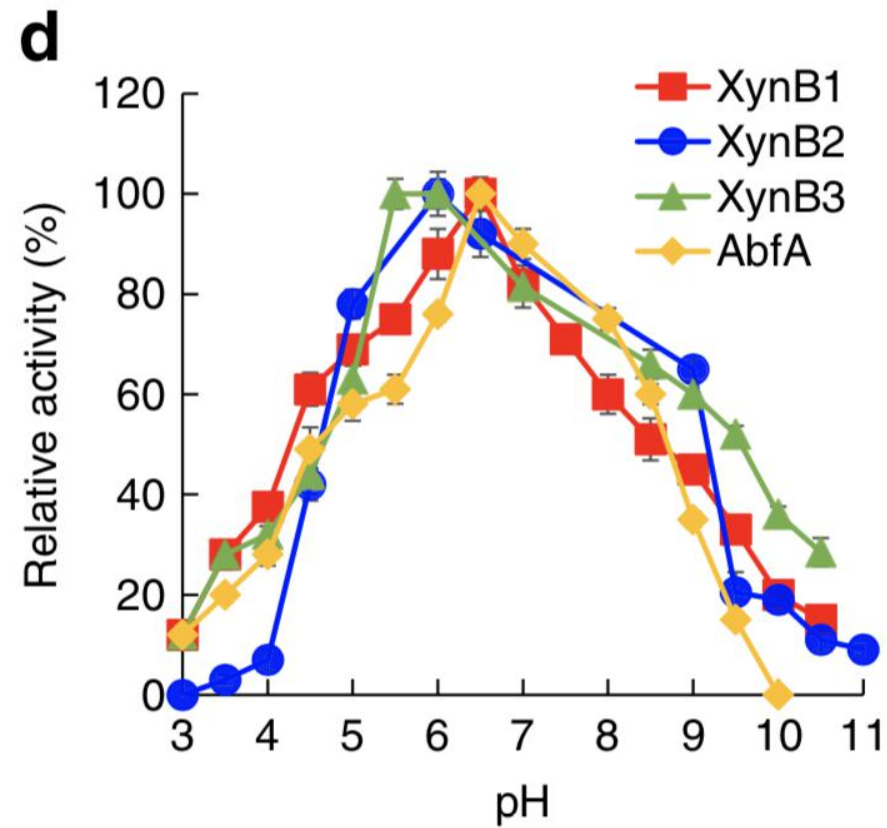
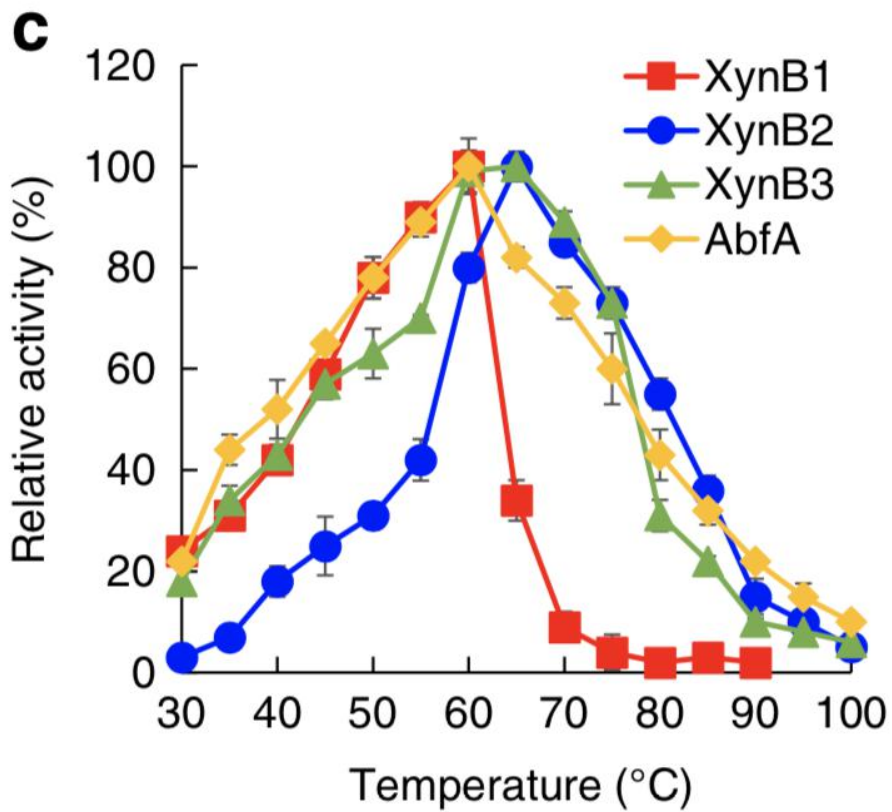
55°C



65°C



75°C



XynB1 60°C
XynB2 65°C
XynB3 65°C
AbfA 60°C

XynB2 4.5-10.4 6.0
XynB1 3.0-10.5 6.5
XynB3 3.0-10.5 5.5
AbfA 3.5-9.5 6.5

Results

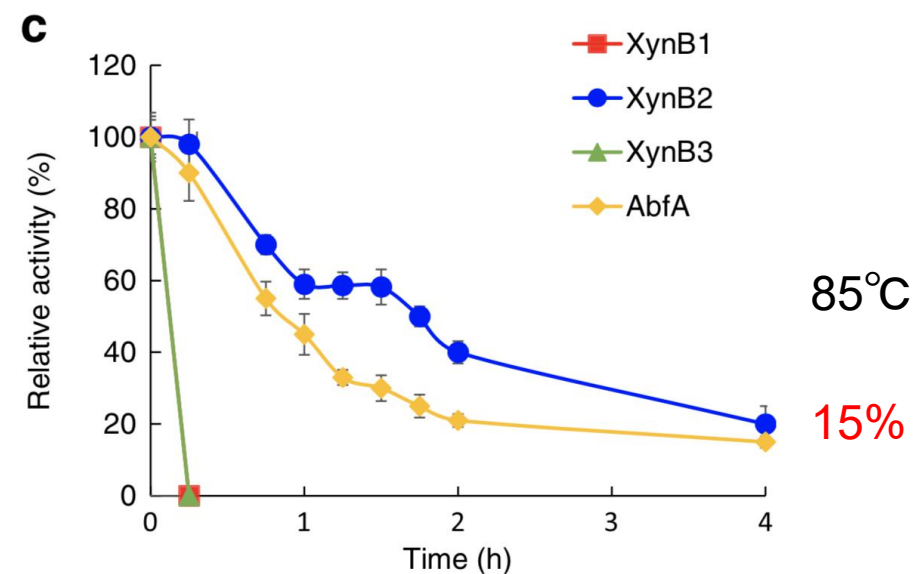
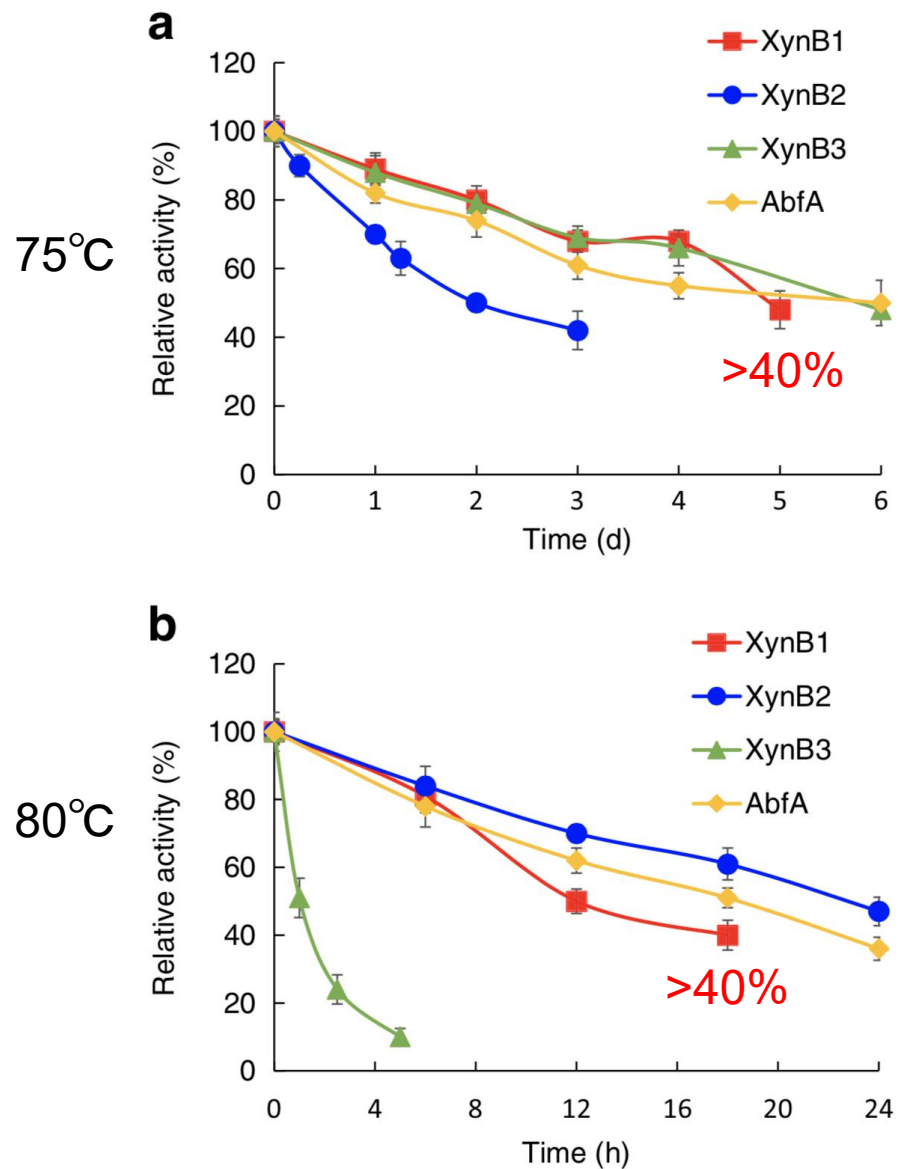


Fig. 5 Thermostability of *XynB1*, *XynB2*, *XynB3*, and *AbfA*. **a** The residual activities when *XynB1*, *XynB2*, *XynB3*, and *AbfA* were individually incubated at 75 °C. **b** The residual activities when *XynB1*, *XynB2*, *XynB3*, and *AbfA* were individually incubated at 80 °C. **c** The residual activities when *XynB1*, *XynB2*, *XynB3*, and *AbfA* were individually incubated at 85 °C

<10min 无活性损失

80°C 6h (XynB1) 8h (XynB2) 30min (XynB3) 80%

5h XynB3 10%

Table 1 Effects of reagents on XynA1, XynA2, XynB1, XynB2, XynB3, and AbfA activities

Reagents	Relative activity (%)					
	XynA1	XynA2	XynB1	XynB2	XynB3	AbfA
None	100.0 ± 7.9	100.0 ± 3.1	100.0 ± 9.2	100.0 ± 5.6	100.0 ± 4.1	100.0 ± 6.8
Al ³⁺ [5 mM]	92.3 ± 5.6	86.5 ± 4.1	98.9 ± 1.1	122.3 ± 10.2	106.6 ± 6.8	96.5 ± 1.6
Ca ²⁺ [5 mM]	165.3 ± 2.0	116.6 ± 8.1	168.8 ± 4.4	125.4 ± 8.4	138.8 ± 3.3	112.5 ± 8.8
Co ²⁺ [5 mM]	20.3 ± 3.5	0	40.8 ± 7.3	96.5 ± 4.8	50.8 ± 1.4	99.8 ± 2.1
Cu ²⁺ [5 mM]	32.2 ± 5.5	0	88.6 ± 3.5	93.2 ± 3.5	69.2 ± 5.9	91.1 ± 3.6
Fe ³⁺ [5 mM]	25.8 ± 6.8	20.1 ± 4.5	89.9 ± 2.1	146.4 ± 6.5	98.9 ± 3.3	92.3 ± 5.0
K ⁺ [5 mM]	145.4 ± 10.1	108.4 ± 3.4	137.7 ± 3.8	102.5 ± 12.9	99.6 ± 12.1	98.8 ± 6.5
Mg ²⁺ [5 mM]	102.2 ± 3.5	106.5 ± 10.6	87.6 ± 6.7	114.7 ± 2.3	86.4 ± 6.8	105.1 ± 3.9
Mn ²⁺ [5 mM]	42.5 ± 1.8	16.9 ± 10.6	92.5 ± 3.8	144.2 ± 6.5	84.9 ± 3.1	106.6 ± 8.8
Na ⁺ [5 mM]	126.6 ± 0.1	105.5 ± 1.8	109.9 ± 1.3	97.9 ± 5.7	96.4 ± 1.6	114.4 ± 10.5
Ni ²⁺ [5 mM]	0	101.8 ± 5.1	17.9 ± 0.1	87.6 ± 7.7	40.8 ± 0.5	88.4 ± 1.1
Zn ²⁺ [5 mM]	140.7 ± 7.8	0	14.8 ± 0.1	65.6 ± 5.3	38.2 ± 0.8	70.3 ± 1.5
DTT [5 mM]	65.5 ± 2.9	85.9 ± 0.6	105.5 ± 1.8	95.6 ± 4.7	92.3 ± 5.8	80.5 ± 2.2
βME [5 mM]	90.3 ± 0.5	73.3 ± 3.3	105.9 ± 5.1	98.8 ± 2.2	92.8 ± 1.1	87.7 ± 2.6
EDTA [5 mM]	103.3 ± 3.5	98.5 ± 1.5	95.6 ± 6.8	104.5 ± 3.7	95.6 ± 8.9	93.3 ± 3.0
SDS [0.5% (w/v)]	0	0	63.6 ± 3.8	77.8 ± 12.6	0	70.3 ± 5.8
Tween 40 [0.5% (v/v)]	0	0	50.6 ± 5.5	87.6 ± 7.7	0	87.9 ± 11.2
Pentane [5% (v/v)]	98.5 ± 2.2	85.9 ± 1.7	100.5 ± 3.3	93.6 ± 2.5	97.8 ± 8.5	90.6 ± 7.4
Decane [5% (v/v)]	82.6 ± 3.9	77.3 ± 6.5	94.3 ± 5.5	90.5 ± 5.0	88.9 ± 4.5	80.9 ± 6.6
Pentadecane [5% (v/v)]	90.3 ± 0.8	88.0 ± 1.2	96.8 ± 3.6	98.8 ± 3.4	90.9 ± 6.5	92.5 ± 6.9
Acetone [5% (v/v)]	90.6 ± 6.6	94.4 ± 2.9	85.9 ± 1.7	81.5 ± 5.6	90.9 ± 3.3	97.8 ± 4.6
Methanol [5% (v/v)]	92.2 ± 1.5	80.4 ± 2.7	89.5 ± 0.9	92.9 ± 6.5	93.6 ± 5.1	85.5 ± 7.7
Ethanol [5% (v/v)]	82.5 ± 8.8	80.3 ± 1.7	85.5 ± 2.5	78.9 ± 3.6	81.0 ± 5.5	86.6 ± 4.2
<i>n</i> -Propanol [5% (v/v)]	85.5 ± 1.8	70.9 ± 2.9	75.5 ± 3.8	83.6 ± 12.5	85.9 ± 8.4	80.6 ± 5.1
<i>n</i> -Butanol [5% (v/v)]	72.7 ± 5.9	80.5 ± 5.7	77.7 ± 7.1	81.3 ± 5.5	77.5 ± 4.9	79.1 ± 7.0

Table 1 Effects of reagents on XynA1, XynA2, XynB1, XynB2, XynB3, and AbfA activities

Reagents	Relative activity (%)					
	XynA1	XynA2	XynB1	XynB2	XynB3	AbfA
None	100.0 ± 7.9	100.0 ± 3.1	100.0 ± 9.2	100.0 ± 5.6	100.0 ± 4.1	100.0 ± 6.8
Al ³⁺ [5 mM]	92.3 ± 5.6	86.5 ± 4.1	98.9 ± 1.1	122.3 ± 10.2	106.6 ± 6.8	96.5 ± 1.6
Ca ²⁺ [5 mM]	165.3 ± 2.0	116.6 ± 8.1	168.8 ± 4.4	125.4 ± 8.4	138.8 ± 3.3	112.5 ± 8.8
Co ²⁺ [5 mM]	20.3 ± 3.5	0	40.8 ± 7.3	96.5 ± 4.8	50.8 ± 1.4	99.8 ± 2.1
Cu ²⁺ [5 mM]	32.2 ± 5.5	0	88.6 ± 3.5	93.2 ± 3.5	69.2 ± 5.9	91.1 ± 3.6
Fe ³⁺ [5 mM]	25.8 ± 6.8	20.1 ± 4.5	89.9 ± 2.1	146.4 ± 6.5	98.9 ± 3.3	92.3 ± 5.0
K ⁺ [5 mM]	145.4 ± 10.1	108.4 ± 3.4	137.7 ± 3.8	102.5 ± 12.9	99.6 ± 12.1	98.8 ± 6.5
Mg ²⁺ [5 mM]	102.2 ± 3.5	106.5 ± 10.6	87.6 ± 6.7	114.7 ± 2.3	86.4 ± 6.8	105.1 ± 3.9
Mn ²⁺ [5 mM]	42.5 ± 1.8	16.9 ± 10.6	92.5 ± 3.8	144.2 ± 6.5	84.9 ± 3.1	106.6 ± 8.8
Na ⁺ [5 mM]	126.6 ± 0.1	105.5 ± 1.8	109.9 ± 1.3	97.9 ± 5.7	96.4 ± 1.6	114.4 ± 10.5
Ni ²⁺ [5 mM]	0	101.8 ± 5.1	17.9 ± 0.1	87.6 ± 7.7	40.8 ± 0.5	88.4 ± 1.1
Zn ²⁺ [5 mM]	140.7 ± 7.8	0	14.8 ± 0.1	65.6 ± 5.3	38.2 ± 0.8	70.3 ± 1.5
DTT [5 mM]	65.5 ± 2.9	85.9 ± 0.6	105.5 ± 1.8	95.6 ± 4.7	92.3 ± 5.8	80.5 ± 2.2
βME [5 mM]	90.3 ± 0.5	73.3 ± 3.3	105.9 ± 5.1	98.8 ± 2.2	92.8 ± 1.1	87.7 ± 2.6
EDTA [5 mM]	103.3 ± 3.5	98.5 ± 1.5	95.6 ± 6.8	104.5 ± 3.7	95.6 ± 8.9	93.3 ± 3.0
SDS [0.5% (w/v)]	0	0	63.6 ± 3.8	77.8 ± 12.6	0	70.3 ± 5.8
Tween 40 [0.5% (v/v)]	0	0	50.6 ± 5.5	87.6 ± 7.7	0	87.9 ± 11.2
Pentane [5% (v/v)]	98.5 ± 2.2	85.9 ± 1.7	100.5 ± 3.3	93.6 ± 2.5	97.8 ± 8.5	90.6 ± 7.4
Decane [5% (v/v)]	82.6 ± 3.9	77.3 ± 6.5	94.3 ± 5.5	90.5 ± 5.0	88.9 ± 4.5	80.9 ± 6.6
Pentadecane [5% (v/v)]	90.3 ± 0.8	88.0 ± 1.2	96.8 ± 3.6	98.8 ± 3.4	90.9 ± 6.5	92.5 ± 6.9
Acetone [5% (v/v)]	90.6 ± 6.6	94.4 ± 2.9	85.9 ± 1.7	81.5 ± 5.6	90.9 ± 3.3	97.8 ± 4.6
Methanol [5% (v/v)]	92.2 ± 1.5	80.4 ± 2.7	89.5 ± 0.9	92.9 ± 6.5	93.6 ± 5.1	85.5 ± 7.7
Ethanol [5% (v/v)]	82.5 ± 8.8	80.3 ± 1.7	85.5 ± 2.5	78.9 ± 3.6	81.0 ± 5.5	86.6 ± 4.2
<i>n</i> -Propanol [5% (v/v)]	85.5 ± 1.8	70.9 ± 2.9	75.5 ± 3.8	83.6 ± 12.5	85.9 ± 8.4	80.6 ± 5.1
<i>n</i> -Butanol [5% (v/v)]	72.7 ± 5.9	80.5 ± 5.7	77.7 ± 7.1	81.3 ± 5.5	77.5 ± 4.9	79.1 ± 7.0

Table 2 Substrate specificities of XynA1, XynA2, XynB1, XynB2, XynB3, and AbfA

Substrates ^a	Relative activity (%)					
	XynA1	XynA2	XynB1	XynB2	XynB3	AbfA
Beechwood xylan	100.0 ± 2.5	100.0 ± 5.1	5.5 ± 1.3	8.4 ± 1.1	6.2 ± 0.5	–
Birchwood xylan	78.3 ± 4.4	66.5 ± 2.6	4.2 ± 0.1	6.9 ± 1.6	5.5 ± 0.8	–
Oat spelt xylan	65.3 ± 5.8	70.3 ± 11.2	3.6 ± 0.8	4.8 ± 0.9	1.5 ± 0.5	4.5 ± 1.2
Corn cob xylan	42.8 ± 4.4	50.2 ± 5.5	1.1 ± 0.5	0.8 ± 0.2	1.5 ± 0.4	1.2 ± 0.5
Water-soluble wheat arabinoxylan	54.9 ± 2.6	50.8 ± 4.5	3.1 ± 0.6	3.1 ± 0.6	3.1 ± 0.6	6.5 ± 2.2
Sugar beet arabinan	10.8 ± 6.9	12.5 ± 4.4	5.6 ± 1.0	5.6 ± 1.0	5.6 ± 1.0	7.8 ± 0.9
Debranched arabinan	6.9 ± 5.1	8.3 ± 3.9	–	–	–	–
<i>p</i> NP-Xyl	–	–	100.0 ± 2.6	100.0 ± 6.2	100.0 ± 4.4	2.5 ± 1.0
<i>p</i> NP-Araf	–	–	26.6 ± 3.2	–	4.1 ± 0.5	100.0 ± 3.9
<i>p</i> NP-Gal	–	–	–	–	–	–
<i>p</i> NP-Glc	–	–	–	12.6 ± 2.5	–	–
<i>p</i> NP-Arap	–	–	–	–	–	–

– not detected

^a Substrate specificities were examined under standard assay conditions with 1% (*w/v*) beechwood xylan, birchwood xylan, oat spelt xylan, corn cob xylan, water-soluble wheat arabinoxylan, sugar beet arabinan, debranched arabinan, and 1 mM of *p*NP-Xyl, *p*NP-Araf, *p*NP-Gal, *p*NP-Glc, and *p*NP-Arap

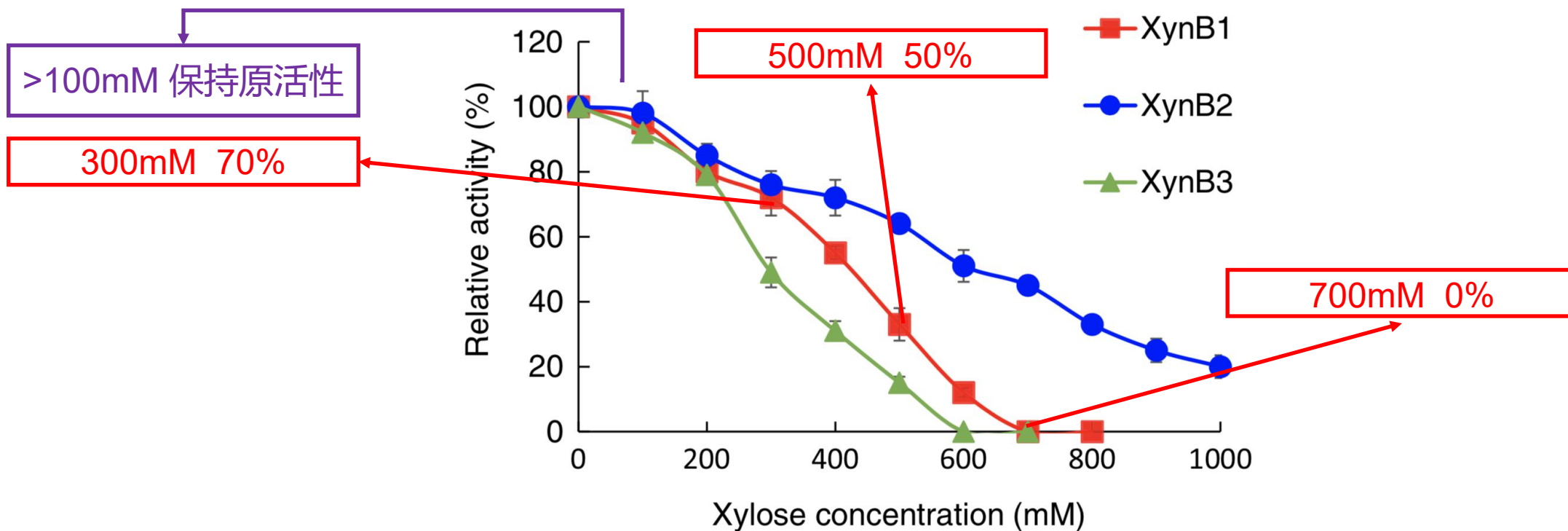


Fig. 6 Effect of xylose on *XynB1*, *XynB2*, and *XynB3* activities. Residual activities were determined when *XynB1*, *XynB2*, and *XynB3* were individually incubated in the presence of different concentrations of xylose

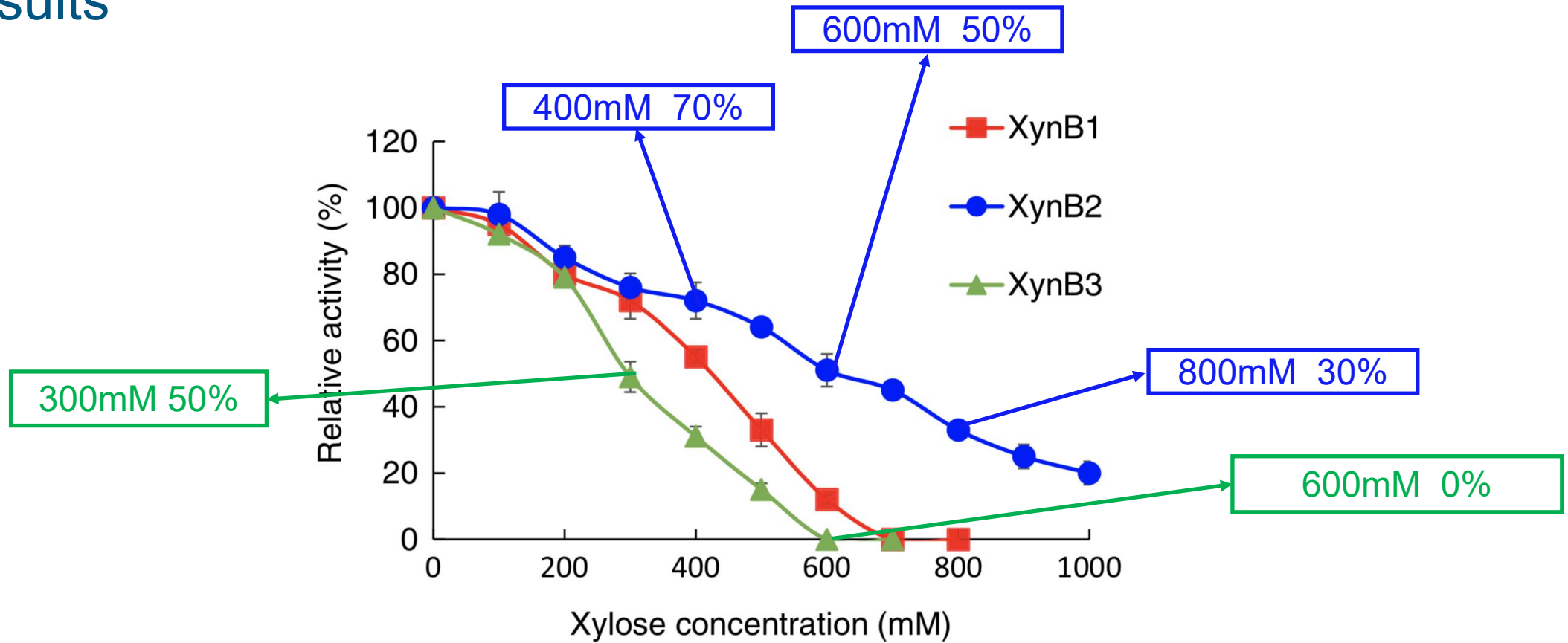


Fig. 6 Effect of xylose on *XynB1*, *XynB2*, and *XynB3* activities. Residual activities were determined when *XynB1*, *XynB2*, and *XynB3* were individually incubated in the presence of different concentrations of xylose

Table 3 Synergistic action of xylanases, β -xylosidases, and α -L-arabinofuranosidase on reducing sugar yields

Enzyme preparations	Oat spelt xylan		Birchwood xylan		Beechwood xylan	
	Reducing sugar yields (mM)	Degree of synergy ^a	Reducing sugar yields (mM)	Degree of synergy	Reducing sugar yields (mM)	Degree of synergy
XynA1	3.359 ± 0.108		6.885 ± 0.133		12.544 ± 0.121	
XynA2	5.514 ± 0.075		5.033 ± 0.093		8.841 ± 0.055	
XynB1	0.036 ± 0.008		0.041 ± 0.011		0.059 ± 0.003	
XynB2	0.065 ± 0.011		0.078 ± 0.007		0.088 ± 0.008	
XynB3	0.022 ± 0.005		0.049 ± 0.008		0.062 ± 0.012	
AbfA	0.068 ± 0.013		–		–	
XynA1 + XynA2	9.368 ± 0.123	1.06	12.968 ± 0.083	1.09*	22.658 ± 0.155	1.06
XynA1 + XynB1	3.674 ± 0.052	1.08*	6.971 ± 0.103	1.01	13.451 ± 0.191	1.06*
XynA1 + XynB2	4.543 ± 0.108	1.33*	10.095 ± 0.029	1.45*	16.039 ± 0.023	1.27*
XynA1 + XynB3	4.024 ± 0.034	1.19*	8.044 ± 0.015	1.16*	14.092 ± 0.016	1.12*
XynA1 + AbfA	4.461 ± 0.009	1.30*	9.561 ± 0.031	1.39*	15.602 ± 0.105	1.24*
XynA2 + XynB1	10.561 ± 0.015	1.90*	6.638 ± 0.073	1.31*	10.015 ± 0.016	1.13*
XynA2 + XynB2	11.104 ± 0.054	1.99*	9.672 ± 0.057	1.89*	9.942 ± 0.020	1.11*
XynA2 + XynB3	10.069 ± 0.024	1.82*	10.066 ± 0.010	1.98*	10.106 ± 0.131	1.14*
XynA2 + AbfA	8.833 ± 0.035	1.58*	7.985 ± 0.031	1.59*	12.311 ± 0.014	1.39*
XynA1 + XynB1 + AbfA	6.589 ± 0.023	1.90*	9.223 ± 0.061	1.33*	18.156 ± 0.033	1.44*
XynA1 + XynB2 + AbfA	7.331 ± 0.055	2.10*	11.316 ± 0.092	1.63*	16.882 ± 0.051	1.34*
XynA1 + XynB3 + AbfA	6.665 ± 0.059	1.93*	9.881 ± 0.012	1.43*	16.536 ± 0.033	1.31*
XynA2 + XynB1 + AbfA	13.991 ± 0.031	2.49*	8.855 ± 0.066	1.75*	14.451 ± 0.011	1.62*
XynA2 + XynB2 + AbfA	12.578 ± 0.022	2.23*	11.744 ± 0.034	2.30*	11.514 ± 0.038	1.29*
XynA2 + XynB3 + AbfA	11.855 ± 0.044	2.12*	12.221 ± 0.019	2.40*	13.150 ± 0.024	1.48*
XynA1 + XynB1 + XynB2 + AbfA	7.085 ± 0.014	2.01*	11.253 ± 0.105	1.61*	19.655 ± 0.083	1.55*
XynA1 + XynB1 + XynB2 + XynB3 + AbfA	6.989 ± 0.029	1.97*	10.386 ± 0.064	1.47*	18.059 ± 0.033	1.42*
XynA2 + XynB1 + XynB2 + AbfA	10.016 ± 0.045	1.76*	9.008 ± 0.038	1.75*	9.896 ± 0.151	1.10*
XynA2 + XynB1 + XynB2 + XynB3 + AbfA	9.124 ± 0.014	1.60*	8.325 ± 0.055	1.60*	9.988 ± 0.104	1.10*

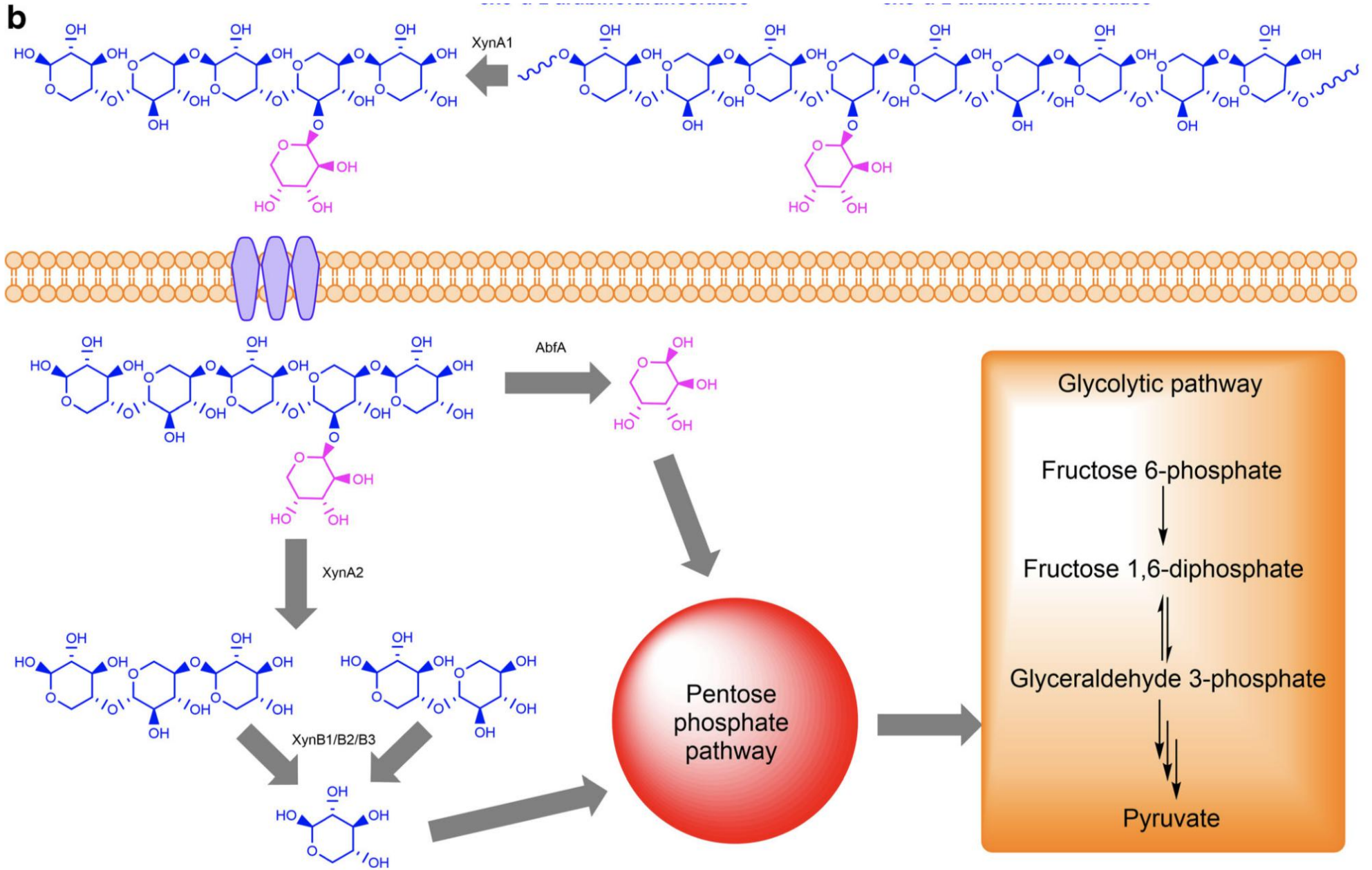
Samples of xylan were incubated in the presence of either single or a combination of endo-xylanase (0.25 U XynA1 or XynA2), β -xylosidase (1.0 U XynB1, XynB2, or XynB3), and α -L-arabinofuranosidase (1.0 U AbfA)

– not detected

*The enzymatic reaction is significantly synergistic at $P < 0.05$ (Tukey's test by OriginPro 8)

^a The degree of synergy is defined as the ratio of xylose equivalents from enzyme reactions to the sum of the xylose equivalents released by the individual enzymes

Discussion



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